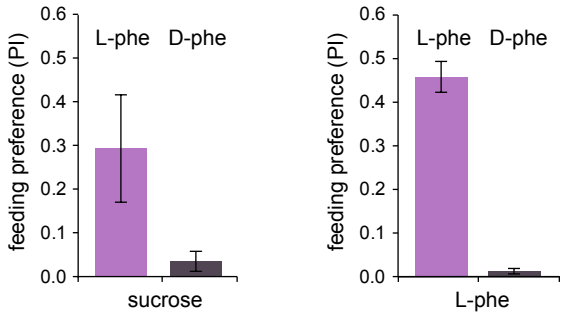
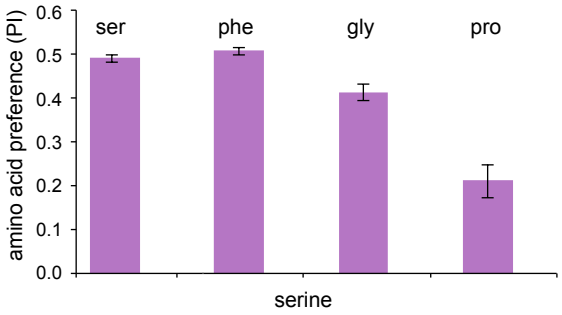


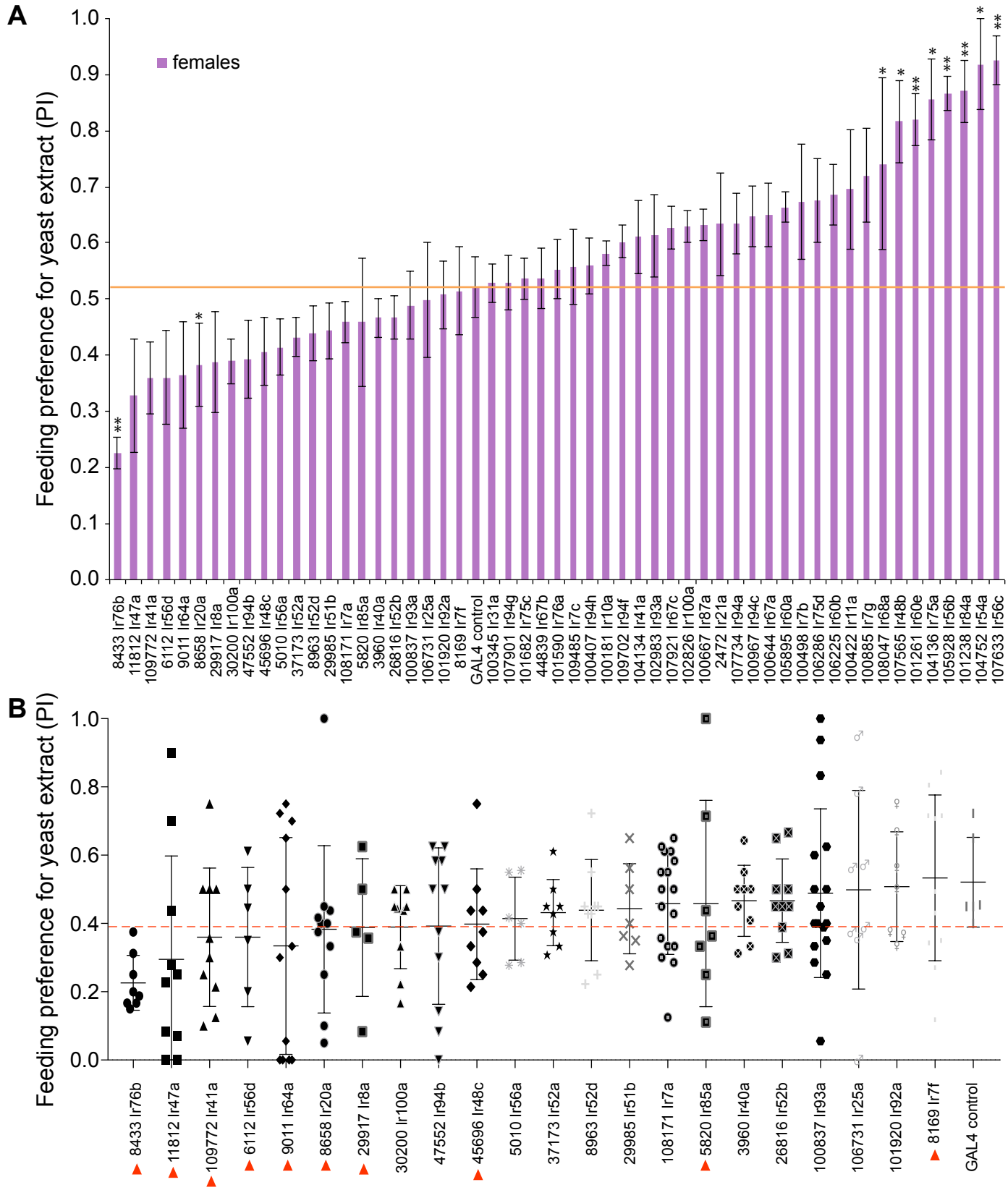
Supplementary Figure 1. Amino acid preference depends on isomeric form, Related to Figure 2.

Mean PI obtained from binary feeding tests with choices between 25 mM of L- or D-phenylalanine (pink dye), as indicated, and 5 mM sucrose (blue dye; left), and from binary feeding assays in which either 25 mM L- or D-phenylalanine, as indicated, were tested with 25 mM L-phenylalanine. Results of pink/blue dye swap conditions were pooled for D-phe/L-phe experiments. *n*=6–9. Results are shown for mated females, genotype was w^{1118}.



Supplementary Figure 2. Amino acid preference depends on identity, Related to Figure 2.

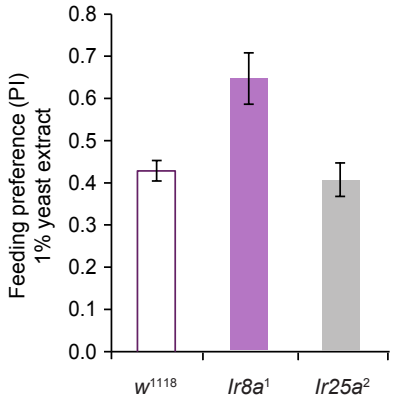
Mean PI obtained from binary feeding tests with choices between 25 mM of the indicated amino acid (pink dye) and 25 mM serine (blue dye). *n*=6–11. Results are shown for mated females, genotype was w^{1118}.



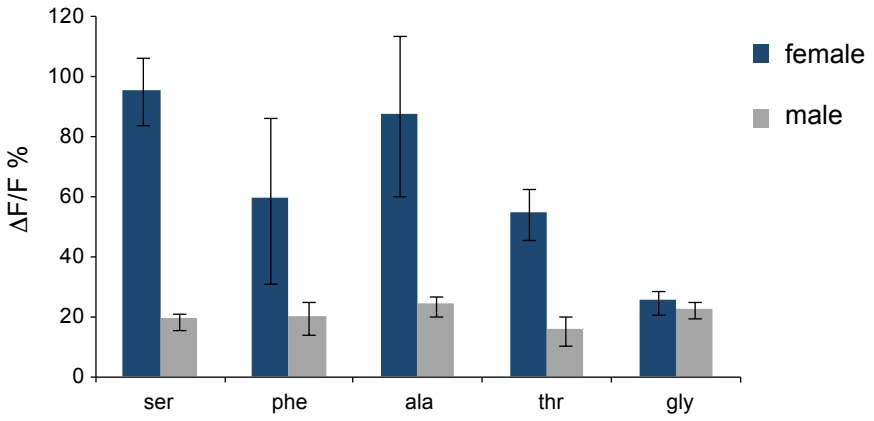
Supplementary Figure 3. An *RNAi* screen to identify *Irs* involved in feeding preference for yeast extract, Related to Figure 3.

(A) Mean PI values of mated females for 1% yeast extract (pink dye) in binary choice tests with 5 mM sucrose (blue dye). Genotypes were *elav-GAL4*/*UAS-Ir-RNAi*; *UAS-Dcr2* or *elav-GAL4*; *UAS-Dcr2*/*UAS-Ir-RNAi*. *Ir* gene name and Vienna *Drosophila RNAi* Canter stock number is listed for each bar. Control flies are *elav-GAL4*/+; *UAS-Dcr2*/+ (GAL4 control). The orange line indicates mean PI of wild type (w^{1118}) females. n=5–19. **P*<0.05, ***P*<0.01, ****P*<0.001, Mann-Whitney *U* tests versus GAL4 control.

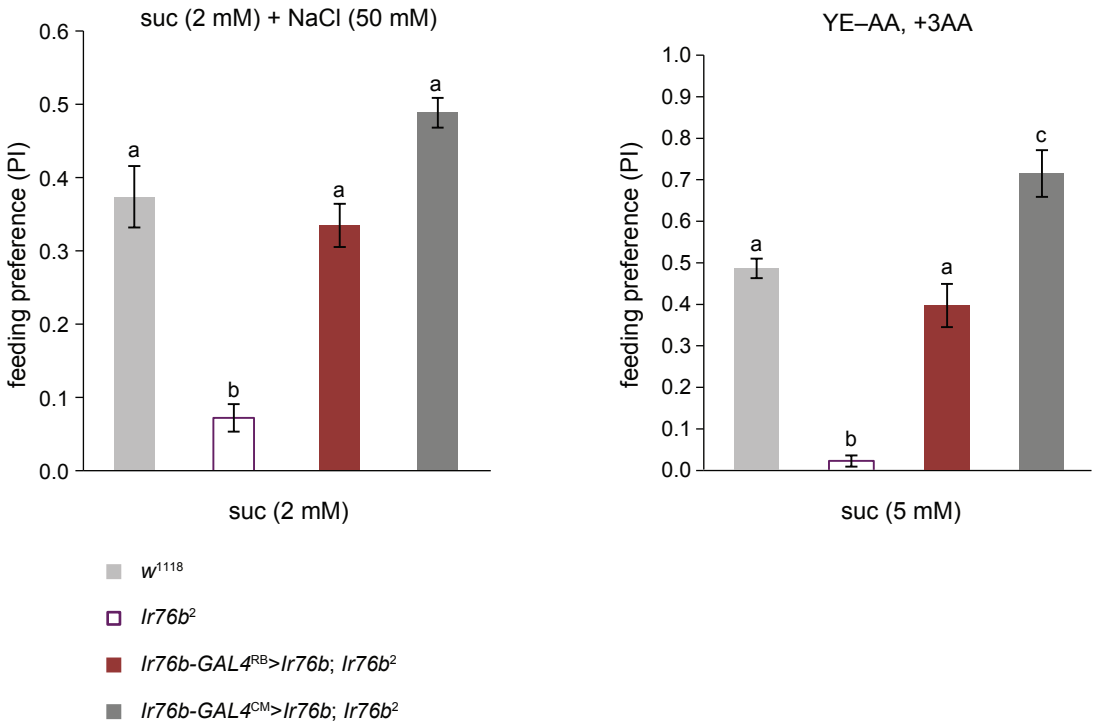
(B) Scatter plot depicting PI values for individual trials for all lines that yielded mean PI values less than that of the GAL4 control. Genotypes were as in (A). Red dashed line indicates value of Mean–S.D. for the GAL4 control. Red arrowheads indicate lines that were chosen for further analysis.



Supplementary Figure 4. Preference for yeast extract is not reduced in *Ir8a* or *Ir25a* mutants, Related to Supplementary Figure 3.
Mean PI of mated females for 1% yeast extract (pink dye) tested against 5 mM sucrose (blue dye) in binary feeding assays. Genotypes were as follows: w^{1118} (wild type), $Ir8a^{1}$; *Bl*/*CyO* ($Ir8a^{1}$) and $Ir25a^{2}$/$Ir25a^{2}$ ($Ir25a^{2}$). *n*=6–10.



Supplementary Figure 5. Amino acid sensitivity in tarsal neurons displays sexual dimorphism, Related to Figure 3.
Mean percent changes in GCaMP3 fluorescence in I*r76b-GAL4* tarsal neurons of female and male flies, as indicated, upon application of individual amino acids. Amino acids were tested at 100 mM, except phenylalanine at 50 mM. *n*=8–36 for females and *n*=3–11 for males. Genotype was w^{1118}.



Supplementary Figure 6. Both *Ir76b-GAL4*^{RB} and *Ir76b-GAL4*^{CM} rescue appetitive response to salt and amino acids, Related to Figure 5.
Mean PI of males and females (pooled) for 50 mM NaCl mixed with 2 mM sucrose (pink dye) tested against 2 mM sucrose (blue dye), and of mated females for 1% yeast extract without amino acids supplemented with 25 mM of serine, phenylalanine and threonine (YE–AA, +3AA, pink dye; tested against 5 mM sucrose, blue dye). Genotypes were as follows: *w*^{1118} (wild type), *Ir76b*^{2}/*Ir76b*^{2} (*Ir76b*^{2}), *Ir76b-GAL4*^{RB}/*Ir76b-GAL4*^{RB}; *Ir76b*^{2}, *UAS-Ir76b*/*Ir76b*^{2}, *UAS-Ir76b* (*Ir76b-GAL4*^{RB}>*Ir76b*; *Ir76b*^{2}), *Ir76b*^{2}, *UAS-Ir76b*/*Ir76b*^{2}, *Ir76b-GAL4*^{CM} (*Ir76b-GAL4*^{CM}>*Ir76b*; *Ir76b*^{2}). *n*=6-18. For each experimental condition, different letters indicate significantly different groups, *P*<0.05, one-way ANOVA with Tukey's *post hoc* test.

SUPPLEMENTAL MATERIAL LEGENDS

Supplementary movie 1. Related to Figure 3. Movie showing GCaMP3 fluorescence in *Ir76b-GAL4* cells in the female foreleg. Control stimulus of water was applied at 15.912–17.544 seconds.

Supplementary movie 2. Related to Figure 3. Movie showing GCaMP3 fluorescence in *Ir76b-GAL4* cells in the female foreleg. 100 mM serine was applied at 13.464–15.096 seconds.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Fly stocks

Ir8a (BL 41744), *Ir25a*^{2} (BL 41737), *Ir76b*^{05} (BL 9824), *Ir76b*^{1} (BL 51309), *Ir76b*^{2} (BL 51310), *Df*(*Ir76b*) (BL 5126), *UAS-mCD8::GFP* (BL 5130), *UAS-Stinger* (BL 29648), *lexAop-mCherry::HA* (BL 52271), *UAS-GCaMP3* (BL 32236), *Gr64f-GAL4* (BL 57669), *Gr89a-GAL4* (BL 57676), and P element transposase (BL 3664) were obtained from the *Drosophila* Bloomington Stock Center. *D. pseudoobscura* flies were obtained from the *Drosophila* Species Stock Center. *UAS-RNAi* flies for the *Ir* family, *SPR*, and *tra*, were obtained from the Vienna *Drosophila* RNAi Center. The following stocks were generously shared by others: *Ir76b-GAL4*^{RB} and *UAS-Ir76b* (Richard Benton, University of Lausanne, Switzerland); *Ir76b-GAL4*^{CM} (Craig Montell, University of California, Santa Barbara); *UAS-Kir2.1* (Kristin Scott, University of California, Berkeley); *fruP1-LexA* (Bruce Baker, Janelia Research Campus).

Generation of Ir76b-LexA transgenic flies

The promoter reported for *Ir76b-GAL4*^{RB} is a 916bp fragment of the sequence immediately upstream of the predicted start codon (Silbering et al., 2011); Primers reported for amplifying the promoter fragment for *Ir76b-GAL4*^{CM} are 5'-GGTTGACCCAGTCTAATGTATGTAATTG and 5'-CGATACGAGTGCCTACTGTACTCTTTAG (Zhang et al., 2013), which yields a 922bp amplicon, also immediately upstream of the predicted start codon. Thus, the two constructs differ in 7 bp at the 5' end. Observed differences in the

expression patterns of the two drivers are possibly due to differences in insertion sites. *Ir76b-LexA*^{RB} was created using a promoter fragment amplified using primer binding sites for 5'-CCAGTCTAATGTATGTAATTG and 5'-CGATACGAGTGCCTACTG. Several independent insertion lines were tested, which showed some variability in expression but the majority showed overlap in expression with *Ir76b-GAL4*^{RB}.

Generation of UAS-Ir transgenic flies

A full-length *AgIr76b* cDNA sequence (VectorBase: AGAP011968) was synthesized by Genescript (Piscataway, NJ); a full-length *Ir20a* coding sequence was amplified from genomic DNA using primers 5'-ATGTTGGCAAGCTTGAA and 5'-TTACAAGCTATTGAAAAATACG. Both were cloned into pUASg-attB and integrated in the attP40 phiC31 landing site.

Generation of Ir20a mutants

Ir20a was targeted using the following oligos: CTTCGGGATTGAAGTATACCAGTG and AAACCACTGGTATACTTCAATCC, which were ligated into pU6-BbsI-chiRNA (Addgene # 45946). The resulting plasmid was directly injected into *vas-Cas9* embryos (BL 51324) and emerging adult flies were crossed with a balancer stock and saved as isogenic lines. The genomic region spanning the CRISPR targeted site was sequenced for each line. Several deletion alleles were recovered.

Feeding preference assays

0-2 day old flies were transferred to fresh food vials (10 males and 10 females per vial), maintained at 25°C with >50% humidity under a 12:12 light:dark cycle, and tested at 5-7 days of age (except for the *Ir76b>Ir20a* experiments in which 7-10 day old flies were tested). Prior to experiments, flies were starved for 24-26 hours in vials with water-saturated Kimwipe beds. Starvation time for *D. pseudoobscura* was 26 hours. This starvation regime was chosen to permit evaluation of innate or baseline preference for various tastants (including amino acids), as opposed to preferences modulated by specific dietary requirements. Tests were performed in tight-fit Petri dishes (Falcon 35-1006). Solutions of 0.75% agarose containing the stimuli and either 0.25 mg ml^{-1} indigo carmine (Sigma I8130) or 0.5 mg ml^{-1} sulforhodamine B (Sigma 230162) were prepared fresh and spotted in equal numbers in the Petri dishes. To account for any possible bias caused by the dyes, tests were typically performed with the same dye/stimulus combinations (as specified in accompanying figure legends). Flies were fed in the Petri dishes for 2 hours at 25°C in a Styrofoam dark humid chamber. Feeding was performed between 2–6 PM, after which the flies were frozen and scored for color in the abdomen. Only trials in which >50% flies survived and >50% participated were included in the analysis. Preference indices were calculated using the following formula: $[N_{pink} + 0.5N_{purple}] / [N_{pink} + N_{blue} + N_{purple}]$.

Tastants

The following tastants were obtained from Sigma: Caffeine (C8960), D-phenylalanine (P17151), Glycine (320331), L-alanine (5129), L-arginine (A8094), L-asparagine (11149), L-aspartic acid sodium salt monohydrate (92384), L-cysteine (30089), L-glutamic acid monosodium salt monohydrate (92834), L-glutamine (98540), L-histidine (53319), L-isoleucine (17403), L-leucine (61819), L-lysine (L5501), L-methionine (64319), L-phenylalanine (P5482), L-proline (81709), L-serine (84959), L-threonine (89179), L-valine (94619), sucrose (S7903), yeast extract (Y1625) and yeast nitrogen base without amino acids and ammonium sulfate (Y1251). Additional tastants were obtained from the following sources: HCl 37% (A.C.S. reagent, 320331); NaCl (Macron Fine Chemicals, 7647-14-5). Tastants were dissolved in water for behavior and calcium imaging experiments, and in 30 mM tricholine citrate (Sigma, T0252) for electrophysiological recordings.

Immunohistochemistry

Fly brains were dissected and fixed in paraformaldehyde and blocked using normal goat serum. Primary antibodies were mouse α-nc82 (1:20, DSHB AB 2314866), rat α-CD8a (1:100, Invitrogen MCD0820), rabbit α-HA (1:100, Abcam ab9110) and chick α-GFP (1:500 or 1:10,000, Abcam ab13970); secondary antibodies were Alexa-488 α-rat (1:150, Invitrogen A11006), Alexa-568 α-mouse (1:150, Invitrogen A11004), Alexa-488 α-chick (1:150, Invitrogen A11039), Alexa-568 α-rabbit (1:150, Invitrogen A11036), and Alexa-647 α-mouse (1:150,

Invitrogen A21235). Confocal *z*-stack images were acquired using a Leica SP5 confocal microscope and analyzed using ImageJ.

Calcium imaging

Flies aged ≥7 days, maintained at 29 °C for ≥4 days (to allow higher expression of *UAS-GCaMP*) were used for imaging. For single fly preparations for imaging, a fly was anesthetized briefly, decapitated, and glued to the base of a tight-fit Petri dish (Falcon 35-1006) using double-sided sticky tape. The sticky tape was also used to secure the forelegs such that the terminal 2-3 segments remain uncovered. A drop of water (100 µL) was used to cover the exposed part of the leg. Tastants were applied by adding 100 µL drops at 2X concentrations to the water drop. Between stimuli, the foreleg was rinsed once with water before addition of a second water drop. GCaMP3 fluorescence was recorded using a Leica SP5 confocal microscope. A filter block with 488 nm excitation filter and 500–535 nm emission filter was used. The focal plane was first adjusted to maximize the number of cell bodies that were visible in the fifth tarsal segment. The gain was reduced such that cell bodies were green in the spectrum log (mean intensity ≤10), after which images were acquired at ~2.5 frames per second using a 10X objective. Stimuli were added ~10–20 seconds after onset of recording, which was continued for ~2 minutes. Images were analyzed using the Leica SP5 LAS AF software (in quantify mode) to obtain heat maps and fluorescence intensity values. ΔF/F % values were calculated separately for each cell body using the mean intensity value of all frames in the 5-second period prior

to addition of the stimulus ($F_{pre(cell)}$) and mean intensity value of all frames in the 5-second period around the peak response ($F_{post(cell)}$). Mean intensity values ($F_{pre(cell)}$ and $F_{post(cell)}$) were calculated similarly for one region of interest chosen in the vicinity of the labeled cell bodies. For wild type analysis, only cell bodies that showed ΔF/F% of ≥10 were included; all cells were included for the experiment in Figure 5A. In all cases, cells with ΔF/F% values that deviated >2 standard deviations from the mean were excluded from the analysis.

ΔF/F% was calculated with the following formula:

$$\frac{[F_{post(cell)} - F_{post(background)}] - [F_{pre(cell)} - F_{pre(background)}]}{[F_{pre(cell)} - F_{pre(background)}]} \times 100$$

SUPPLEMENTAL REFERENCES

Silbering, A.F., Rytz, R., Grosjean, Y., Abuin, L., Ramdya, P., Jefferis, G.S., and Benton, R. (2011). Complementary function and integrated wiring of the evolutionarily distinct *Drosophila* olfactory subsystems. J Neurosci *31*, 13357-13375.

Zhang, Y.V., Ni, J., and Montell, C. (2013). The molecular basis for attractive salt-taste coding in *Drosophila*. Science *340*, 1334-1338.